EFFECT OF p-BROMOPHENACYL BROMIDE, AN INHIBITOR OF PHOSPHOLIPASE A_2, ON ARACHIDONIC ACID RELEASE AND PROSTAGLANDIN SYNTHESIS BY THE GUINEA-PIG UTERUS *in vitro*

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1 The synthesis of prostaglandins $F_{2\alpha}$ and E_2 by guinea-pig uterine homogenates was inhibited by p-bromophenacyl bromide (PBPAB), an inhibitor of phospholipase A_2.

2 Metabolism of prostaglandin $F_{2\alpha}$ by uterine homogenates was undetectable; this was not affected by PBPAB.

3 There was no significant difference between the amounts of arachidonic acid released from uterine homogenates on days 7 and 15 of the oestrous cycle. Small amounts of dihomo-γ-linolenic acid were detected in the homogenates.

4 The release of arachidonic acid from uterine homogenates was greatly inhibited by PBPAB.

5 Addition of exogenous arachidonic acid to uterine homogenates did not overcome the inhibition of uterine prostaglandin $F_{2\alpha}$ synthesis produced by PBPAB.

6 It is concluded that PBPAB inhibits both the release of arachidonic acid from the guinea-pig uterus and its subsequent conversion into prostaglandins.

Introduction

Prostaglandin synthesis *in vivo* occurs in several steps. The non-steroidal, anti-inflammatory drugs inhibit the conversion of the precursor fatty acids, such as arachidonic acid, into prostaglandins (Vane, 1971). It is generally recognized that the levels of free arachidonic acid in tissues are too low for any significant production of prostaglandins, and that arachidonic acid is released from some source in order for prostaglandin synthesis to occur. Membrane phospholipids have been regarded for several years as a possible source, the arachidonic acid being released by the action of phospholipase A_2 (Lands & Samuelsson, 1968; Vogt, Meyer, Kunze, Lufft & Babilli, 1969; Bartels, Kunze, Vogt & Wille, 1970; Kunze & Vogt, 1971; Flower & Blackwell, 1976). Consequently, inhibition of phospholipase A_2 activity should prevent prostaglandin synthesis by tissues.

p-Bromophenacyl bromide (PBPAB) is an inhibitor of porcine pancreatic phospholipase (Volwerk, Pieterson & de Haas, 1974). It alkylates the imidazole side-chain of the histidine residue at position 53 of the phospholipase A_2 molecule, which contains 120 amino acids in all. A compound which inhibits the release of arachidonic acid from phospholipids, without preventing the conversion of the acid into prostaglandins, would be very useful in studying pro-

staglandin synthesis by tissues and in elucidating the source of the arachidonic acid. Consequently we have studied the effect of PBPAB on prostaglandin synthesis by, and arachidonic acid release from, the guinea-pig uterus *in vitro*. This tissue is known to synthesize and release prostaglandin $F_{2\alpha}$ under physiological conditions *in vivo* (see Poyser, 1976) and to synthesize predominantly prostaglandin $F_{2\alpha}$ with some prostaglandin E_2, from endogenous precursors when homogenized and incubated *in vitro*, (Poyser, 1972).

Methods

Effect of PBPAB on prostaglandin $F_{2\alpha}$ synthesis by the guinea-pig uterus

Guinea-pigs in dioestrus (vagina closed) were used in groups of four. Prostaglandin synthesizing capacity of the uterus is lower during dioestrus than at the end of the oestrous cycle when the vagina is open (Poyser, 1972). The 4 guinea-pigs in each group were killed by stunning and incising the neck. The uterus from each animal was removed and cut into four equal parts. Quarters from different animals were combined in such a way as to reconstitute one 'uterus' (i.e. upper

right horn, upper left horn, lower right horn and lower left horn). This resulted in four uterine samples containing one quarter of the uterus from each animal. Each sample was weighed, and was usually in the range of 0.6 to 0.8 grams. The uterine tissue was opened longitudinally and cut into small pieces. Each sample was placed in 14.5 ml Krebs solution (composition (g/l): NaCl 6.9, KCl 0.354, $CaCl_2$ 0.282, $MGSO_4$, $7H_2O$ 0.294 g, KH_2PO_4 0.162, $NaHCO_3$ 2.1, glucose 2.0), which had been pre-aerated with 5% CO_2 in O_2. To three of the samples was added PBPAB, dissolved in 0.5 ml ethanol so calculated to give a known final concentration. To the fourth sample was added only 0.5 ml ethanol, and this sample acted as the control. Each sample was incubated in the Krebs solution, aerated with 5% CO_2 in O_2, at 37°C for 30 minutes. Following this period, the uterine tissue was homogenized in the incubating fluid, using a Fisons' glass homogenizer, and then incubated for another 90 min under the same conditions.

The reaction was stopped by acidifying to pH 4.5 with 1 N HCl and immediately extracting the prostaglandins with ethyl acetate. (The pH of the homogenates following incubation was always in the range of 7.4 to 7.6). The amounts of prostaglandin $F_{2\alpha}$ formed were measured in one of two ways: (1) Initially, the ethyl acetate extracts were further purified, the different prostaglandins separated by column chromatography, and the prostaglandin $F_{2\alpha}$ isolated assayed biologically on the rat fundal strip, by methods described previously, (Poyser, 1972). The identity of the prostaglandin $F_{2\alpha}$ was confirmed by gas chromatography-mass spectrometry. No prostaglandin $F_{1\alpha}$ was detected. (2) The later samples were assayed by radio-immunoassay using an antibody raised and tested in our laboratory (Blatchly & Poyser, 1974; Naylor & Poyser, 1975; Dighe, Emslie, Henderson, Rutherford & Simon, 1975). The double antibody method was used, and two additional cross-reactivity studies were performed. 13,14-Dihydro-15-keto-prostaglandin $F_{2\alpha}$ cross-reacted 0.56% and prostaglandin D_2 cross-reacted 17.1%. Previous studies had failed to detect prostaglandin D_2 in the uterus, so the relatively high cross-reactivity of this prostaglandin with the $F_{2\alpha}$ antibody was not considered to invalidate the assay.

The ethyl acetate extracts of the incubates were evaporated to dryness and each residue dissolved in 20 ml ethyl acetate. Each solution was then diluted 2 or 4 times with ethyl acetate, and aliquots of 10, 20, 50 and 100 µl were assayed in duplicate. Parallelism (i.e. a similar value obtained for the amount of prostaglandin $F_{2\alpha}$ in the original extract irrespective of volume assayed) was obtained for all samples. This provides good evidence that prostaglandin $F_{2\alpha}$ was being assayed, as substances likely to cross-react do not have standard curves parallel to that of prostaglandin $F_{2\alpha}$. (Prostaglandin $F_{1\alpha}$ is an exception, but

the synthesis of prostaglandin $F_{1\alpha}$ by guinea-pig uterine homogenates has not been detected, for reasons apparent later). Consequently, the amount of prostaglandin $F_{2\alpha}$ in each extract was taken as the mean of the 8 values obtained.

Comparable results were obtained by both methods of assay. The amount of prostaglandin $F_{2\alpha}$ synthesized by 100 mg of uterine tissue was calculated for each sample. The percentage inhibition of prostaglandin $F_{2\alpha}$ synthesis produced by PBPAB was established for each group of four guinea-pigs.

Effect of PBPAB on the metabolism of prostaglandin $F_{2\alpha}$ by the guinea-pig uterus

The uterus of a guinea-pig in dioestrus was removed and each uterine horn divided in half. The top half of one horn was combined with the bottom half of the other horn, and each uterine sample was weighed. The uterine tissue was opened longitudinally and cut into small pieces. One sample was placed in 14.5 ml Krebs solution to which was added PBPAB, dissolved in 0.3 ml ethanol, to give a final concentration of 100 µg/ml. The other sample was placed in 14.5 ml Krebs solution to which was added 0.3 ml ethanol. Both samples were incubated at 37°C for 30 min, aerated with 5% CO_2 in O_2, then homogenized in the incubation fluid. To each homogenate was added 0.4 µCi [9-³H]-prostaglandin $F_{2\alpha}$ (sp.act. 7.5 Ci/mmol, New England Nuclear) dissolved in 0.2 ml methanol. The homogenates were incubated for another 90 min, and the prostaglandins and any metabolites formed extracted with ethyl acetate. Each extract was then further purified by dissolving in 20 ml 67% ethanol and partitioning twice with 20 ml petroleum ether (b.p. 60°–80°C). The ethanol fraction was evaporated to dryness. The residue was dissolved in a mixture of 0.1 ml methanol and 0.1 ml toluene and spotted on to a thin-layer chromatography plate of silica gel (20 cm × 5 cm; Merck). The plate was developed in two solvent systems: the FVI system of Andersen (1969) and the GCM system of Millar (1974). The plates were scanned with a Panax thin-layer scanner RTLS-1A to locate the radioactive spots. A plate containing authentic [9-³H]-prostaglandin $F_{2\alpha}$ was also run. This experiment was performed on two guinea-pig uteri.

Effect of PBPAB on prostaglandin E_2 synthesis by the guinea-pig uterus

The effect of PBPAB on uterine prostaglandin E_2 synthesis was only examined in the later stages of this study when a reliable and sensitive method of measuring low levels of prostaglandin E_2 became available. An antibody to prostaglandin E_2 was purchased from the Pasteur Institute, Paris, where the antibody had been raised and tested (Dray,

Charbonnel & Maclouf, 1975). In our double-antibody method, the antibody had the cross-reactivities listed in Table 1. The working range of the assay was from 3 to 80 pg prostaglandin E_2. The coefficient of variation for within assays (the intra-assay precision) was 13.3% calculated from 354 samples (of guinea-pig, sheep and human uterine extracts) in 12 assays. The inter-assay precision was calculated from the results obtained by assaying in duplicate at least two different aliquots of a standard solution containing 1 ng/ml prostaglandin E_2. The coefficient of variation between assays was 9.9%.

Accuracy was assessed in two ways. Firstly, the mean ($\pm$ s.e. mean) of the values obtained from the 12 assays for the standard solution of 1 ng/ml prostaglandin E_2 was 0.96 ± 0.2 ng/ml. Secondly, a guinea-pig uterus was homogenized and incubated as described previously (Poyser, 1972). Prostaglandins were extracted and the amount of prostaglandin E_2 formed was assayed before and after silicic acid column chromatography. A control column, on which 20 ng prostaglandin E_2 was placed, was run in parallel to correct for procedural losses incurred during the chromatography step. The values obtained for the amount of prostaglandin E_2 formed were 29.3 ng when assayed before chromatography and 29.2 ng (corrected for a recovery of 61.5% from the control column) when assayed after chromatography. The amount of prostaglandin E_2 produced by 100 mg tissue was, therefore, 4.9 ng when assayed by both techniques. This value is in the expected range (Poyser, 1972).

Consequently, the antibody could measure prostaglandin E_2 accurately, and with sufficient precision, using ethyl acetate extracts of guinea-pig uterine homogenates. The amounts of prostaglandin E_2 synthesized by the guinea-pig uterine homogenates in the absence and presence of PBPAB (100 µg/ml) were determined in a similar manner to that of prostaglandin $F_{2\alpha}$ using radioimmunoassay. Parallelism was again achieved providing further evidence that prostaglandin E_2 was being assayed in each sample.

Table 1   Cross-reactivities of several prostaglandins and prostaglandin metabolites with the prostaglandin E_2 antibody

Prostaglandin	% Cross-reactivity
PGE_1	15.1
$PGF_{2\alpha}$	0.17
PGA_2	0.14
PGB_2	0.05
15 Keto-PGE_2	0.58
13,14 Dihydro-15 keto-PGE_2	0.06

Release of arachidonic acid from the guinea-pig uterus at times of low and high prostaglandin synthesis

Five guinea-pigs were killed on day 7 of the oestrous cycle (low prostaglandin synthesis) and day 15 (high prostaglandin synthesis) and their uteri removed. Each uterus was divided into four equal parts, and the top half of one uterine horn was combined with the bottom half of the opposite horn. The two uterine samples were weighed. One sample was then immediately homogenized in 15 ml ice-cold Tyrode solution (composition (g/l): NaCl 8.0, KCl 0.2, CaCl$_2$ 0.2, MgCl$_2$ 0.1, NaH$_2$PO$_4$ 0.05, NaHCO$_3$ 1.0, glucose 1.0), which contained indomethacin (50 µg/ml). To the homogenate was added 0.02 µCi [^{14}C]-arachidonic acid (sp.act. 58 mCi/mmol; Amersham) and the lipids extracted as described below. The second sample was homogenized in 15 ml Tyrode solution containing indomethacin and the homogenate incubated for 60 min at 37°C, aerated with O$_2$. [^{14}C]-arachidonic acid (0.02 µCi) was then added, the pH lowered to 4.5 with 1 N HCl and the lipids extracted by partitioning three times with 30 ml ethyl acetate. The ethyl acetate fractions were combined and evaporated to dryness. The residue was dissolved in 20 ml 67% ethanol and the fatty acids extracted by partitioning three times with 20 ml petroleum ether (b.p. 60°–80°C). The petroleum fractions were combined and evaporated to dryness. Arachidonic acid was separated from other fatty acids in the extract by reversed-phase partition chromatography using lipidex 5000 (Becker-Delft, Holland) with a solvent system of methanol, water, 1,2-dichloroethane and glacial acetic acid in the ratio of 800:200:100:1.1 (G. Jones, personal communication). The ^{14}C-label enabled the tracing of arachidonic acid on the column, and a correction to be made for procedural losses following isolation (average recovery = $67.6 \pm 1.3\%$, $n = 33$). The samples of arachidonic acid isolated were assayed by gas chromatography using a Pye series 104 chromatograph containing a column of 4% OV1 on supelcoport or 5% SP 2330 on chromasorb W. 2,3-Benzofluoroanthene was employed as an internal standard. In several instances the identity of the arachidonic acid was confirmed by gas chromatography-mass spectrometry. The amounts of free arachidonic acid released per 100 mg tissue before and after incubation were then calculated.

Two further guinea-pigs were examined for the presence of dihomo-γ-linolenic acid in the uterus in a similar manner, using [^{14}C]-dihomo-γ-linolenic acid as the tracer.

Effect of PBPAB on arachidonic acid release from the uterus

Five guinea-pigs were killed on day 7 of the oestrous

cycle and the uterus removed. Each uterus was divided into six equal parts, and one-third of one uterine horn combined with one-third of the opposite horn obtained from a different region. The three uterine samples were weighed. One sample was immediately homogenized in ice-cold Krebs solution containing indomethacin (50 µg/ml) and the arachidonic acid content measured as just described. The uterine tissue in the other two samples was opened longitudinally, then cut into small pieces. One sample was placed in Krebs solution containing indomethacin (50 µg/ml), whilst the other sample was placed in Krebs solution containing indomethacin (50 µg/ml) and PBPAB (100 µg/ml) (all samples contained 0.6 ml ethanol, used for dissolving the compounds). These samples were incubated for 30 min at 37°C, aerated with 5% CO_2 in O_2. Both samples were then homogenized and incubated under the same conditions for a further 60 minutes. Arachidonic acid content was then estimated as before.

Effect of exogenous arachidonic acid and PBPAB on prostaglandin $F_{2\alpha}$ synthesis by the uterus

Five groups of 4 guinea-pigs were used. The guinea-pigs were in dioestrus. The uteri from the animals in each group were removed and divided into four equal parts. One quarter of each uterus was combined so as to reconstitute one 'whole uterus' in the manner previously described. The four uterine samples were weighed, and the uterine tissue opened longitudinally and cut into small pieces. One sample from each group was incubated for 30 min at 37°C, in 15 ml of one of the following solutions: (1) Krebs solution (control) (2) Krebs solution containing 100 µg/ml PBPAB. (3) Krebs solution containing 10 µg/ml arachidonic acid. (4) Krebs solution containing 100 µg/ml PBPAB and 10 µg/ml arachidonic acid.

All samples contained an equal amount of ethanol (0.6 ml). The arachidonic acid had been previously purified by reversed-phase partition chromatography by Dr G. Jones.

After the 30 min incubation, each sample was homogenized in its incubation fluid and further incubated under the same conditions for 90 minutes. The amounts of prostaglandin $F_{2\alpha}$ synthesized were determined by radioimmunoassay.

Results

Effect of PBPAB on prostaglandin $F_{2\alpha}$ synthesis by the guinea-pig uterus

Prostaglandin $F_{2\alpha}$ synthesis by homogenates of guinea-pig uteri was inhibited by PBPAB (Figure 1). Increasing the concentration by PBPAB increased the
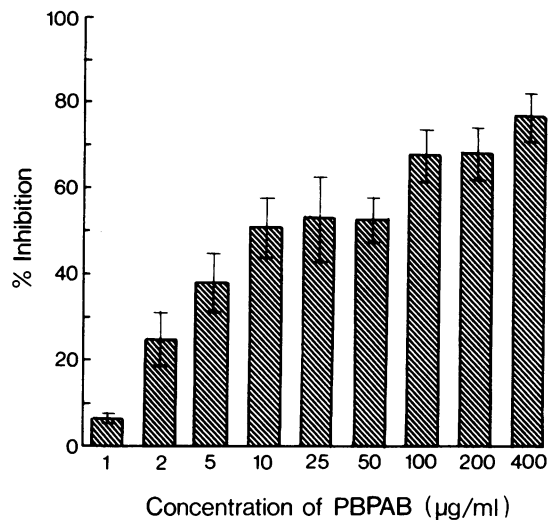

Figure 1 Effect of *p*-bromophenacyl bromide (PBPAB) on prostaglandin $F_{2\alpha}$ synthesis by homogenates of guinea-pig uteri.

inhibition of synthesis, although two plateaux of inhibition were seen. The maximum inhibition of synthesis obtained was 70 to 80%. The concentration of PBPAB, could not be increased because of its limited solubility.

Effect of PBPAB on metabolism of prostaglandin $F_{2\alpha}$ by the guinea-pig uterus

Previous studies have shown that, under the conditions used, there is no detectable metabolism of prostaglandin $F_{2\alpha}$ by the guinea-pig uterus, even at different stages of the cycle (Maule Walker, 1975). This study confirmed that observation in so far as all the thin layer chromatography plates had only one spot containing radioactivity at R_F values between 0.43 and 0.45. The R_F value of [9-^{3}H]-prostaglandin $F_{2\alpha}$ was 0.43. PBPAB does not, therefore, affect metabolism of prostaglandin $F_{2\alpha}$ by the uterus, and metabolism remained undetectable.

Effect of PBPAB on prostaglandin E_2 synthesis by the uterus

At the one concentration of PBPAB studied (100 µg/ml), protaglandin E_2 synthesis by guinea-pig uterine homogenates was inhibited by $63.0 \pm 4.3\%$ (mean $\pm$ s.e., $n = 5$). The absolute amounts of prostaglandin E_2 synthesized were considerably less than the amounts of $F_{2\alpha}$ synthesized, confirming earlier studies (Poyser, 1972; Maule Walker & Poyser, 1974).

Release of arachidonic acid from the guinea-pig uterus at times of low and high prostaglandin synthesis

The results are shown in Table 2. The incubation of guinea-pig uterine homogenates for 60 min significantly increased ($P < 0.01$) the levels of free arachidonic acid detectable on both day 7 and day 15. There was no significant difference between the levels on day 7 (low prostaglandin synthesizing capacity) and day 15 (high prostaglandin synthesizing capacity) when compared before or after incubation.

In the two additional guinea-pig uteri studied, the presence of dihomo-γ-linolenic acid following incubation was just detectable, but was not measurable. There was about 1% or even less of the amount of arachidonic acid present.

Effect of PBPAB on arachidonic acid release from the uterus

The results are shown in Table 3. The pre- and post-incubation (without PBPAB) levels of arachidonic acid are not significantly different from those in Table 2. The addition of PBPAB ($100 \mu g/ml$) to the homogenate, however, reduced the amount of arachidonic acid released following incubation by 75.9%.

Table 2 Amounts of arachidonic acid (mean $\pm$ s.e.) in guinea-pig uterine homogenates before and after incubation ($n = 5$)

| | Amount of arachidonic acid ($\mu g/100$ mg tissue) | |
	Day 7	Day 15
Before incubation	2.1 ± 0.4^a	1.7 ± 0.1^a
After incubation	5.1 ± 0.4^b	5.0 ± 1.0^b

Values with the same superscript are not significantly different.

Table 3 Effect of *p*-bromophenacyl bromide (PBPAB, $100 \mu g/ml$) on arachidonic acid release from guinea-pig uterine homogenates ($n = 5$).

	Amount of arachidonic acid ($\mu g/100$ mg tissue) (mean $\pm$ s.e.)
Non-incubated	1.6 ± 0.4^a
Incubated	4.5 ± 0.4^b
Incubated with PBPAB	2.3 ± 0.4^a

Values with the same superscript are not significantly different.

Effect of exogenous arachidonic acid and PBPAB on prostaglandin $F_{2\alpha}$ synthesis by the uterus

This experiment was designed to investigate whether adding exogenous arachidonic acid could overcome the inhibition of uterine prostaglandin $F_{2\alpha}$ synthesis produced by PBPAB. The results are shown in Table 4. It is apparent that adding arachidonic acid to the incubate did not overcome the inhibitory action of PBPAB. Furthermore, the addition of exogenous arachidonic acid did not increase uterine prostaglandin $F_{2\alpha}$ synthesis, in samples without PBPAB, above control levels. This last observation confirms earlier studies (Poyser, 1971).

Discussion

A compound which inhibits phospholipase A_2 without preventing the conversion of precursor fatty acids into prostaglandins would be very useful in the overall study of prostaglandin synthesis, especially in establishing the source of the arachidonic acid. This study has shown that PBPAB, an inhibitor of porcine pancreatic phospholipase A_2 (Volwerk *et al.*, 1974), inhibits the synthesis of prostaglandin $F_{2\alpha}$ from endogenous precursors by the guinea-pig uterus, when homogenized and incubated *in vitro*. The results obtained are not due to a redirection of synthesis towards prostaglandin E_2 as its synthesis was also inhibited by PBPAB. Also, the results are not due to an increase in metabolism induced by PBPAB, as metabolism remained undetectable. These results do not show whether the inhibition of prostaglandin synthesis was due to a lack of arachidonic acid in the incubate or to an inhibition of its conversion into prostaglandins.

Consequently, the effect of PBPAB on arachidonic acid release from the guinea-pig uterus was also examined. It was found that this compound did reduce quite markedly the increase in level of free arachidonic acid which occurs during the incubation of uterine

Table 4 Effect of exogenous arachidonic acid ($10 \mu g/ml$) on the inhibition of uterine prostaglandin $F_{2\alpha}$ synthesis produced by *p*-bromophenacyl bromide (PBPAB, $100 \mu g/ml$) ($n = 5$).

Treatment	% Inhibition (mean + s.e.)
Control homogenates	—
Homogenates + arachidonic acid	$6.2 + 4.6$
Homogenates + PBPAB	$74.0 + 4.1$*
Homogenates + PBPAB and arachidonic acid	$63.6 + 5.8$*

* not significantly different.

homogenates. Whether this is due to it solely inhibiting phospholipase A_2 has still to be established. There are other sources of arachidonic acid besides phospholipids, such as triglycerides and cholesterol esters. Consequently, an inhibition of triglyceride lipases and cholesterol esterase may also contribute to the lowering in the level of free arachidonic acid.

It is apparent that the level of arachidonic acid remaining after incubation of the uterus with PBPAB is still far in excess of the amounts (usually 40 to 150 ng/100 mg tissue) of prostaglandin $F_{2\alpha}$ synthesized. Furthermore, the addition of exogenous arachidonic acid did not overcome the inhibition of prostaglandin $F_{2\alpha}$ synthesis produced by PBPAB. This compound therefore inhibits both the release of arachidonic acid from uterine tissue and the conversion of arachidonic acid into prostaglandins. PBPAB is only suitable for determining the source of arachidonic acid if one measures the levels of this acid and does not rely on prostaglandin synthesis as the end-point.

Several drugs have been investigated for their ability to inhibit phospholipase A_2. Local anaesthetics and tranquillisers prevent the release of arachidonic acid from phospholipids, but they also, with the exception of amethacine, inhibit the conversion of arachidonic acid into prostaglandins (Seppälä, Saris & Gauffin, 1971; Scherphof, Scarpa & van Toorenenbergen, 1972; Kunze, Bohn & Vogt, 1974; Kunze, Bohn & Bahrke, 1975). Propranolol also prevents the release of arachidonic acid from phospholipids (Seppälä et al., 1971). However, these drugs do not inhibit phospholipase A_2 directly like PBPAB. Earlier evidence suggested they interacted with Ca^{2+} (Scherphof et al., 1972; Waite & Sisson, 1972) although later evidence suggests they interact with the phospholipids (Scherphof & Estenberg, 1975). Vane and his co-workers have used mepacrine as a phospholipase A inhibitor (Vane, 1976), though this drug, like other antimalarials, is a good inhibitor of triglyceride lipases (Markus & Ball, 1969). It also inhibits prostaglandin synthetase (Flower & Blackwell, 1976) so mepacrine would also appear unsuitable to use as a specific phospholipase A_2 inhibitor. Perhaps amethacine should be investigated further.

An interesting finding in the present study concerns arachidonic acid release from uterine homogenates following incubation. There was no difference in the amounts released between day 7 of the oestrous cycle (low prostaglandin synthesis) and day 15 (high prostaglandin synthesis). Consequently, on day 7 there is about 1% conversion of the available arachidonic acid into prostaglandins, which increases to about 2 to 3% by day 15. It follows that limitation of prostaglandin synthesis on day 7 is not due to lack of precursor. It is therefore not surprising that the addition of exogenous arachidonic to day 7 uterine homogenates did not increase prostaglandin $F_{2\alpha}$ synthesis.

The increase in synthesis on day 15 is due to an increase in the amount of prostaglandin synthetase in the uterus. Its level is apparently controlled by oestradiol, as the treatment of ovariectomized guinea-pigs with oestradiol increases the prostaglandin $F_{2\alpha}$ synthesizing capacity of the uterus (Naylor & Poyser, 1975). Oestradiol output from the ovary does increase towards the end of the oestrous cycle (Joshi, Watson & Labhsetwar, 1973). Similar findings have been reported for the rat uterus concerning the control of synthetase levels (Ham, Cirillo, Zanetti & Kuehl, 1975).

The precursor of the l-series prostaglandins (e.g. prostaglandins $F_{1\alpha}$ and E_1) dihomo-γ-linolenic acid, was just detectable in uterine homogenates following incubation. There was probably 100 times more of the arachidonic acid present. Consequently, it is not surprising that neither prostaglandin $F_{1\alpha}$ nor E_1 have been detected in extracts of guinea-pig uterine homogenates.

The levels of free arachidonic acid following homogenization of the uterus but before incubation were remarkably high. It has been reported for the rat brain that there is a rapid release of arachidonic acid during the 10 min period following death (Wolfe, Pappius & Marion, 1976). This may have occurred in the uterus especially as the time of death to the extraction of arachidonic acid was 10 to 15 minutes. Consequently improved techniques of stopping enzyme action at the time of death will have to be used to establish the true levels of free arachidonic acid. Furthermore, it may prove difficult to establish from where the free 1 to 3% of the total arachidonic acid is derived for prostaglandin synthesis even with the use of specific enzyme inhibitors, if indeed there is a specific source.

Another problem which may be difficult to solve is the actual place where arachidonic acid release and prostaglandin synthesis takes place in the intact uterus, in vivo. Both may occur in the endometrium or in the myometrium, or there may even be an interaction between the two sites. Prostaglandin synthesis is likely to take place in the endometrium since its chemical destruction prolongs the oestrous cycle in the guinea-pig (Butcher, Chu & Melampey, 1962). So far, it has proved extremely difficult to separate the guinea-pig uterus into the endometrium and myometrium in vitro, so as to resolve this point.

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